

We claim:

1. A method for improving production of vanillin in cultured *Vanillin planifolia*, which comprises:

5 a) providing a tissue culture of said *Vanilla planifolia*; and

b) supplementing the culture with a compound selected from the group consisting of malic acid, 3,4-dihydroxybenzaldehyde, citric acid, pyruvic acid,
10 oxaloacetic acid, succinic acid, glycosylated lysozyme, and any combination thereof, in an amount effective to improve the vanillin production as compared with cultures not supplemented with the compound.

15 2. The method of claim 1, wherein the tissue culture is an embryo culture.

3. The method of claim 1, wherein the culture is supplemented with malic acid at a concentration of between
20 about 0.01% and 5% by weight of the culture medium.

4. The method of claim 3, wherein the culture is subjected to mechanical shear stress for 21 days, followed by addition of the malic acid at a concentration of between
25 about 1% and 3% by weight of the culture medium.

10087714-022800

5. The method of claim 1, wherein the culture is supplemented with 3,4-dihydroxybenzaldehyde at a concentration of between about 0.1 and 5 mM.

5

6. The method of claim 1, wherein the culture is supplemented with about 0.01 to about 5% by weight of a compound selected from the group consisting of succinic acid, oxaloacetic acid, citric acid and pyruvic acid.

10

7. The method of claim 1, wherein the culture is supplemented with about 1 to about 100 $\mu\text{g/ml}$ of a glycosylated lysozyme elicitor.

15

8. Cultured *Vanilla planifolia* cells, produced by the method of claim 1.

20

9. The cultured *Vanilla planifolia* cells of claim 8, which produce at least twice as much vanillin as equivalent cultured cells not supplemented with the compounds.

25

10. The cultured *Vanilla planifolia* cells of claim 8, which produce at least ten times as much vanillin as equivalent cultured cells not supplemented with the compounds.

11. A method for improving production of vanillin in cultured *Vanilla planifolia*, which comprises:

a) providing an embryo culture of said *Vanilla planifolia*; and

b) subjecting the culture to a stress condition selected from the group consisting of heat stress and mechanical shear stress, in an amount and for a time effective to improve the vanillin production as compared with cultures not subjected to the stress condition.

12. The method of claim 11, wherein the heat stress comprises maintaining the cultures between about 33 and 37°C for between three and seven days.

13. The method of claim 11, wherein the mechanical shear stress is imposed by placing the cultures in an impeller-driven incubator, under conditions whereby the shear stress is caused.

14. Cultured *Vanilla planifolia* cells, produced by the method of claim 11.

15. The cultured *Vanilla planifolia* cells of claim 14, which produce at least twice as much vanillin as equivalent cultured cells not subjected to the stress.

5 16. A method for improving vanillin production in *Vanilla planifolia*, which comprises genetically engineering the *Vanilla planifolia* to overproduce one or more enzymes associated with one or more steps of vanillin biosynthesis in the *Vanilla planifolia*, the steps selected from the group
10 consisting of: chain shortening of p-coumaric acid to p-hydroxybenzaldehyde; chain shortening of ferulic acid to vanillin; hydroxylation of p-hydroxybenzyl alcohol to 3,4-dihydroxybenzyl alcohol or aldehyde; and methylation of 3,4-dihydroxybenzaldehyde to vanillin.

15 17. The method of claim 16, wherein the enzymes are selected from the group consisting of: at least one p-hydroxybenzaldehyde synthase; at least one cytochrome p450 monooxygenase; and at least one methyl transferase.

20 18. The method of claim 16, wherein the enzyme comprises SEQ ID NO:2 or a functional variant thereof.

25 19. The method of claim 18, wherein the enzyme is encoded by SEQ ID NO:1.

10087714.022802

20. The method of claim 16, wherein the genetically engineered *Vanilla planifolia* is a cell or tissue culture.

21. The method of claim 16, wherein the genetically
5 engineered *Vanilla planifolia* is a whole plant.

22. A genetically engineered *Vanilla planifolia* cell
produced by the method of claim 16.

10 23. The cell of claim 22, which produces at least
twice as much vanillin as does an equivalent cell which is
not comparably genetically engineered.

24. A genetically engineered *Vanilla planifolia* plant,
15 regenerated from the cell of claim 22.

25. The plant of claim 24, which produces at least
twice as much vanillin as does an equivalent plant which is
not comparably genetically engineered.

20

26. A method for improving vanillin accumulation in
cell or tissue culture of *Vanilla planifolia*, which
comprises inhibiting production or activity of vanillyl
alcohol dehydrogenase in cells comprising the cell or tissue

10087744-022802

culture, the inhibition resulting in the improved vanillin accumulation.

27. The method of claim 26, wherein the inhibiting
5 comprises genetically engineering the cells to inhibit
expression of a gene encoding the vanillyl alcohol
dehydrogenase.

28. A genetically engineered *Vanilla planifolia* cell
10 or tissue culture produced by the method of claim 27.

29. The method of claim 26, wherein the inhibiting
comprises treating the culture with an inhibitor of vanillyl
dehydrogenase activity.

30. A method for improving vanillin production and
15 accumulation in a *Vanilla planifolia* cell or tissue culture,
which comprises:

a) genetically engineering the *Vanilla planifolia*
20 to overproduce one or more enzymes associated with one or
more steps of vanillin biosynthesis in the *Vanilla*
planifolia, the steps selected from the group consisting of:
chain shortening of p-coumaric acid to p-
hydroxybenzaldehyde; chain shortening of ferulic acid to
25 vanillin; hydroxylation of p-hydroxybenzyl alcohol to 3,4-

1008744-02200

dihydroxybenzyl alcohol or aldehyde; and methylation of 3,4-dihydroxybenzaldehyde to vanillin, thereby resulting in the improved vanillin production; and

- b) inhibiting production or activity of vanillyl
5 alcohol dehydrogenase in cells of the culture, thereby
resulting in the improved vanillin accumulation.

31. A *Vanilla planifolia* cell or tissue culture
produced by the method of claim 30.